RETENTION OF PERCEPTUAL GENERALIZATION OF FEAR EXTINCTION

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Abstract

Fear reduction obtained during a fear extinction procedure can generalize from the extinction stimulus to other perceptually similar stimuli. Perceptual generalization of fear extinction typically follows a perceptual gradient, with increasing levels of fear reduction the more a stimulus resembles the extinction stimulus. The current study aimed to investigate whether perceptual generalization of fear extinction can be observed also after a retention interval of 24 hours. Fear was acquired to three geometrical figures of different size (CS^+, $CS1^+$ and $CS2^+$) by consistently pairing them with a short-lasting suffocation experience (US). Three other geometrical figures that were never followed by the US served as control stimuli (CS^-, $CS1^-$, $CS2^-$). Next, only the CS^+ was extinguished by presenting it in the absence of the US. One day later, fear responses to all stimuli were assessed without any US-presentation. Outcome measures included startle blink EMG, skin conductance, US expectancy, respiratory rate and tidal volume. On day 2 spontaneous recovery of fear was observed in US expectancy and tidal volume, but not in the other outcomes. Evidence for the retention of fear extinction generalization was present in US expectancy and skin conductance, but a perceptual gradient in the retention of generalized fear extinction could not be observed.

Key words: fear extinction, retention, perceptual gradient, psychophysiology, respiration

1. Introduction

Fear conditioning implies that an initially innocent stimulus becomes a predictor (conditioned stimulus – CS) for a biologically relevant aversive event (an unconditioned stimulus – US) because of an experienced contingency between both. As a result of this associative learning process not only the US evokes a fear response (UR- unconditioned response), but also the CS generates a preparatory, defensive response (conditioned response – CR, Domjan, 2005). Once a CR is established it can occur also to other stimuli that never have been paired directly with the US (generalized stimuli – GSs), because they share certain properties with the CS (e.g. Vervliet and Geens, 2014; Lissek et al., 2008; Dunsmoor et al., 2009). Experimental research has demonstrated that physical or conceptual resemblance of the GSs with the CS promotes fear generalization to the GSs (Hajcak et al., 2009; Vervoort et al., 2014). Perceptual generalization seems to vary along a continuum of perceptual similarity: the more a GS resembles a CS, the greater the CR (Lissek et al., 2008).

Fear learning and fear generalization can be very adaptive mechanisms (Dunsmoor et al., 2009) but the capacity to re-evaluate a stimulus as safe when it no longer predicts danger, is just as crucial (Lommen et al., 2013). When the CS is administered repeatedly without the US, the CS-US contingency decreases and the CR gradually declines. It was first assumed that such extinction procedure generates a form of *un*-learning, i.e. the gradual weakening of the CS-US connection (Rescorla and Wagner, 1972; McConnell, 2014). However, several return-of-fear phenomena have demonstrated that the original CS-US fear association is not erased following an extinction procedure (Myers and Davis, 2007). Laboratory studies and clinical practice have systematically documented return of fear and relapse phenomena, respectively (Vervliet et al., 2013). For example, the

mere passage of time can partly reinstall the CR to a previously extinguished CS ('spontaneous recovery'). Also the phenomenon of 'renewal' demonstrates the context dependency of extinction learning (Vervliet et al., 2013).

It is now widely accepted that extinction is not a mere *un*-learning of the excitatory CS-US association, but encompasses a form of new learning (Bouton, 2002; Myers et al., 2006) through which a CS acquires an inhibitory CS-noUS connotation next to the already existing excitatory CS-US association. The efficacy of exposure therapy should thus be evaluated in the light of its ability to create strong and easily retrievable inhibitory CS-noUS memory traces that can outweigh the excitatory fear connection (Raio et al., 2014). Thus, what is learned during exposure therapy is ideally transferrable to contexts other than the therapeutical setting, to stimuli beyond those used in exposure therapy, and over time. However, whereas acquired fear is prone to generalize over contexts, stimuli and time (Vervliet et al., 2013), generalization of fear extinction seems more difficult to establish. With regard to the generalization of fear extinction over different stimuli, early studies of Pavlov indicated that CSs of different sensory modalities that were trained with the same US all evoked less fear after only one of them had been extinguished (Myers and Davis, 2007). This result was however not replicated in an animal study (Kasprow, Schachtman, Cacheiro, & Miller, 1984) that failed to show extinction to a stimulus that had been paired with the same US as an extinction stimulus. More recent experiments suggest that extinction effects are rather 'extinction cue' - specific (Vervliet et al., 2004, 2005; Vervoort et al., 2014). For example, in a series of experiments Vervliet et al. (2004, 2005) demonstrated that only extinction with the original CS, but not with a GS, promotes generalization of fear extinction over stimulus dimensions. Vervoort et al. (2014) replicated these findings but with conceptual instead of perceptual stimulus categories. Interestingly, several studies

(Myers and Davis, 2007; Bass and Hull, 1934; Hovland, 1937) have documented generalization gradients of fear extinction, with the smallest CRs to the extinction cue and increasingly greater CRs to cues falling farther away along the similarity continuum. A hiatus in extant human research is that perceptual generalization gradients of fear extinction have almost always been studied immediately after the extinction procedure, while especially their retention is of clinical relevance. To our knowledge, only one, old study (Hovland, 1937) aimed to investigate the retention of extinction gradients in humans. The results of this study are however flawed by a lack of a good control condition, problems with the basic design and poor statistical analysis.

The purpose of this experiment is therefore to study the perceptual gradient of fear extinction with a panic-relevant US after a retention interval of one day. To this end, we will first install fear to three similar geometrical figures of different size (CS^+, $CS1^+$, $CS2^+$) by reinforcing them 100% with a breathing occlusion (US – see Pappens et al., 2014). Control stimuli will consist of other geometrical figures of similar size (CS^-, $CS1^-$, $CS2^-$). We will subsequently extinguish only one of the three figures (CS^+) by administering unreinforced CS trials. One day later we will test fear responses to all stimuli.

After a successful acquisition and extinction phase, we expect the following effects to occur:

(1) Spontaneous Recovery.

a. Early on in the <u>test</u> phase (test1), the extinguished differential effect ($CS^+ > CS^-$) will recover. That is, the differential effect will be higher during test (test1) compared to the end of extinction (ext2): $(CS^+_{ext2} - CS^-_{ext2}) < (CS^+_{test1} - CS^-_{test1})$

b. Spontaneous recovery will also be visible in a higher response to CS^+ than to CS^- early on in the test phase: $CS^+_{test1} > CS^-_{test1}$.

(**2) Retention of generalization of fear extinction**.

a. At the start of the test phase, the differential effects for the unextinguished CS1 and CS2 pairs will be reduced compared to the differential effect for the CSs at the start of the extinction phase: $[(CS1^+_{test1} - CS1^-_{test1})$ and $(CS2^+_{test1} - CS2^-_{test1})] < (CS^+_{ext1} - CS^-_{ext1})$.

b. We will also test whether the unextinguished CS^+s still evoke stronger fear responses than their control stimuli early on in the test phase: $(CS1^+_{test1}, CS2^+_{test1}) > (CS1^-_{test1}, CS2^-_{test1})$. The more retention of generalization of fear extinction, the less the difference should be between the reinforced and the unreinforced CS1 and CS2.

(3) Perceptual gradient of generalization of fear extinction. During the first block of the test phase (test1), differential responses will be smaller for the CS1 compared to the CS2 pair, because $CS1^+$ is perceptually closer to the extinguished CS^+ than $CS2^+$: $(CS1^+_{test1} - CS1^-_{test1}) < (CS2^+_{test1} - CS2^-_{test1})$.

2. Material and Methods

2. 1 Participants

Thirty-nine first year psychology students (9 men; 18-26 years; *M*=19,87) participated in return for 15 euros/hour of participation.

Exclusion criteria were: current or past history of cardiovascular disease, chronic or acute respiratory disease, pregnancy, current or past history of drug or alcohol abuse or dependence, psychotropic drug use and any current or past psychiatric disorder including panic and anxiety disorder. Participants were instructed to abstain from alcohol and caffeinated drinks 24 hours before the study and from food and drinks two hours before the study. The study protocol was approved by the Medical Ethical

Committee in accordance with the Declaration of Helsinki; all subjects signed an informed consent form stating – amongst other information – that participation was voluntary and that they could withdraw from the study at any moment.

2.2 Stimuli and Apparatus

2.2.1 Stimuli

2.2.1.1 Geometrical figures.

Blue- or yellow colored pictures of geometrical shapes (three triangles and three circles of different size) served as conditional stimuli (CSs). Circle 1 had a diameter of 5.25 cm; circle 2 of 10.5 cm and circle 3 of 21 cm. Triangle 1 consisted of a base of 3.6 cm and 4.2 cm height, the base of triangle 2 measured 7,2 cm with 8,4 cm height and the dimensions of triangle 3 were 14,4 cm base and 16,7 cm height.

2.2.1.2 Breathing occlusion.

The unconditional stimulus (US) was a complete obstruction of the breathing circuitry, making it impossible for the participant to breathe for a period of time. The length of the breathing occlusion was individually tailored by taking 40% of the personal breath holding time (BHT) after expiration.

2.2.2 Breathing apparatus.

A mouthpiece was mounted onto a bacterial filter that was fitted on a pneumotachograph (Fleisch No. 2, Epalinges, Switzerland). The pneumotachograph was attached to a non-rebreathing valve of which the inspiratory port was connected to a 3-way Y-valve (stopcock type) using a vinyl tube (inner diameter: 3.5 cm; length 100 cm). This set-up enabled easy switching between unrestricted breathing and the breathing

occlusion. The signal from the pneumotachograph was amplified using a pressure transducer (Sine Wave Carrier Demodulator CD15, Valydine Engineering™) and was calibrated daily with a 1 liter syringe. Fractional end-tidal CO_2 ($FetCO_2$) was measured using an infrared capnograph (POET II, Criticare, USA) that sampled expired air from the breathing circuit close to the mouthpiece. The capnograph's output was calibrated daily using a calibration gas containing 7.5% CO_2. Airflow and CO_2 waveforms were digitized at 20 Hz.

2.2.3 Skin conductance.

Electrodermal activity was recorded with Fukuda standard Ag/AgCl electrodes (1 cm diameter) filled with a Unibase electrolyte and attached to the hypothenar palm of the non-dominant hand, which was cleaned with tap water before the start of the procedure. The inter-electrode distance was 2.5 cm. A Coulbourn skin conductance coupler (LabLinc v71-23) provided a constant 0.5 V across electrodes. The signal was digitized at 10 Hz.

2.2.4 Eyeblink startle response.

Orbicularis oculi electromyographic activity (EMG) was recorded as an index of the eyeblink component of the startle response with three Ag/AgCl Sensormedics electrodes (0.25 cm diameter) filled with electrolyte gel. After cleaning the skin to reduce inter-electrode resistance, electrodes were placed on the left side of the face (Blumenthal et al., 2005). The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75–04; 13 Hz–500 Hz). The signal was rectified online and smoothed by a Coulbourn multifunction integrator (LabLinc v76–23 A) with a time constant of 50 ms. The EMG signal was digitized and stored at 1000 Hz

from 500ms before the onset of the auditory startle probe (a 95 dB burst of white noise with a rise time < 1 ms presented binaurally for 50 ms through headphones) until 1000 ms after probe onset.

2.2.6 US expectancy dial.

Participants were asked to continuously rate their expectancy of the US with a custom built dial (Pappens et al., 2012). The scale on which they had to rate US-expectancy, ranged from zero ("I am certain that the breathing occlusion is not coming now") to one hundred ("I am certain that the breathing occlusion is coming now"). The dial produced an analogue signal, which was digitized and stored at 10 Hz.

2.2.7 Software.

All devices were connected to a PC through a NI PCI-6221 16-Bit National Instruments data acquisition card (National Instruments, Austin, Texas). Affect 4.0 software (Spruyt et al., 2010) was used for stimulus presentation and data acquisition. Physiological signals were treated off-line with PSPHA (De Clerck et al., 2006), a modular script-based program to generate and apply calibration factors and to extract parameters from each of the recorded signals.

2.3 Procedure

On both days, prior to the experimental procedure, participants were asked to sit quietly and an 8 min resting baseline measurement of ECG was performed. As the ECG-baseline results are beyond the scope of this paper, we will not further discuss them here.

Day 1: Upon arrival, participants received an informed consent containing information on the experiment. The document stated that during the experiment we would measure psychophysiological and self-report responses to different stimuli, amongst which a breathing occlusion, and geometrical figures displayed on a monitor in front of them. We informed them that their task during the experiment consisted of learning to predict when the breathing occlusion would occur and that the geometrical figures might be related to the (non)-occurrence of the occlusion. We stated that participation was voluntary and could be stopped at any moment; that all collected data was confidential; and that data of only one individual would never be shared.

Next, the experimenter measured their post-expiratory BHT by asking them to exhale and then hold their breath for as long as possible. Next, she attached the electrodes required to measure electrodermal and eyeblink startle responses, and put on the headphones. The experimenter also demonstrated how to use the online US expectancy dial. Participants were then asked to put on the nose clip and to start breathing through the mouthpiece. After a short instruction on how to breathe and swallow most comfortably through the mouthpiece, the experimenter left the room and started up the experiment.

At the start of the experiment, 10 acoustic startle probes were administered with a variable inter-stimulus interval (ITI; 12-15 s) to habituate the eyeblink startle response. Next, the acquisition phase followed, in which all 6 geometrical figures were presented 4 times each. In half of the participants all circles were reinforced with a breathing occlusion and none of the triangles – for the other half only the triangles were reinforced. Participants were counterbalanced across two color/shape combinations for

the geometrical figures (blue circles/yellow triangles versus blue triangles/yellow circles).

A reinforced trial consisted of a 10 s baseline, an 8 s presentation of the CS followed by a breathing occlusion of individually tailored duration (40 % of the post-expiratory BHT with a minimum of 8 s), and a variable ITI interval (between 27-30s). Unreinforced trials consisted of a 10s baseline, an 8 s CS and a variable ITI (27-30 s + duration US).

The acquisition phase was immediately followed by an extinction phase in which the largest or smallest (semi-randomized) triangle and circle were administered 8 times each without reinforcement.

An extinction trial consisted of a 10 s baseline, an 8 s CS-presentation followed by a variable ITI (27-30s).

Day 2: Upon arrival, the experimenter explained that the participant had to perform the same task as the day before: they had to learn to predict when the breathing occlusion would be administered. Furthermore she said that they could use the knowledge they had acquired the day before.

After the administration of 10 acoustic startle probes to habituate the eyeblink startle response the test phase was started. In this phase all circles and triangles were administered non-reinforced 4 times each (24 trials).

A trial during the test phase consisted of a 10 s baseline, an 8 s CS-presentation followed by a variable ITI (27-30s).

On both days, we administered 3 startle probes in each trial: during the CS and the US between 5 -7s following stimulus onset; in unreinforced, extinction- or test trials at the moment of the US-absence (5-7s following CS offset) and between 10 - 12 s of the ITI.

At the end of the experiment participants were debriefed.

2.4 Response Definition, Scoring and Statistical Analysis

2.4.1 US-expectancy

In order to create two blocks per phase (acquisition, extinction, and test phase), online US-expectancy ratings were averaged across 2 (acquisition and test) or 4 (extinction) trials. We calculated difference scores between the last and the first second of the CS for each stimulus size (CS, CS1, CS2) and stimulus type (plus, minus) per block and per phase. As such, these difference scores reflect the change in US-expectancy caused by the CS.

2.4.2 Eyeblink startle

Eyeblink startle (EMG) responses were calculated by taking the difference between the peak value in the 21 - 175 ms time window and the mean value from the 0 - 20 ms time window following probe onset. All startle responses (of day 1 and day 2) were T-transformed within persons to correct for interindividual variability that was unrelated to the experimental conditions of interest. For the acquisition , extinction and test phase, startle responses were averaged over 4 trials, per stimulus type (plus, minus), per probe (CS, ITI) and per stimulus size (CS, CS1, CS2). This resulted in 1 block

for the acquisition and the test phases, and in two blocks for extinction. Finally, difference scores were calculated between CS and ITI startle responses of the same block. These CS – ITI difference scores indicate the degree of startle potentiation in the presence relative to the absence of the CS.

2.4.3 Skin conductance

Skin conductance responses (SCRs) were calculated by subtracting the mean skin conductance level (SCL) during baseline (2s before the CS onset) from the maximum SCR during the subsequent 8s CS period. SCR-data were then $LOG_{10}(1 + SCR)$-transformed. In order to create two blocks for each phase (acquisition, extinction, and test phase) SCRs were averaged across 2 (acquisition and test) or 4 (extinction) trials.

2.4.4 Respiratory rate (RR) and Tidal Volume (Vt)

Averages of respiratory rate (RR) and tidal volume (Vt, the average of inspiratory and expiratory volume) were calculated for each 20 sec baseline period prior to the CS and for each 8 sec window corresponding to the CS presentation. As some breaths fell only partially into the baseline or CS window, each breath was weighted for its relative time spent in the time window of interest (baseline, CS). Weighted averages of RR and Vt per time window (baseline, CS) were further averaged across 2 (acquisition and test) or 4 (extinction) trials, yielding two blocks for each phase (acquisition, extinction, and test phase) for each stimulus size (CS, CS1, CS2) and stimulus type (plus, minus). Finally, baseline-blocks were subtracted from CS-blocks; these difference score thus reflect the change in RR or Vt from baseline to CS.

A small proportion of startle and skin data (less than 5%) were missing due to artifacts. Missing data were replaced by taking the mean of the two most adjacent similar trials. Data of 3 participants were removed from the respiratory analyses due to technical problems.

All a priori hypotheses were analyzed one-tailed with planned comparisons. An α-level of .05 was set for statistical significance. All statistical analyses were performed with Statistica Version 12.

2.4.5 Manipulation check

A necessary condition for the study of (a gradient of) retention of fear extinction is that (1) fear acquisition and (2) fear extinction were successfully established in the first place.

2.4.5.1 Fear Acquisition.

We expected that:

a. Differential fear learning would occur in late acquisition (acq2), with higher fear responses to the reinforced ($^+$) than to the unreinforced ($^-$) stimuli: (CS^+_{acq2}, $CS1^+_{acq2}$, $CS2^+_{acq2}$) > (CS^-_{acq2}, $CS1^-_{acq2}$, $CS2^-_{acq2}$).

b. We also expected differential conditioning effects at the end of acquisition for every $^+/^-$ pair separately: $CS^+_{acq2} > CS^-_{acq2}$; $CS1^+_{acq2} > CS1^-_{acq2}$; $CS2^+_{acq2} > CS2^-_{acq2}$

c. A prerequisite for an unambiguous testing of hypotheses 2-3 is also that comparable effects are being established to all $^+/^-$ pairs during acquisition. Therefore, we tested whether the effects established in acquisition were different between the three CS-pairs: ($CS^+_{acq2} - CS^-_{acq2}$) = ($CS1^+_{acq2} - CS1^-_{acq2}$) = ($CS2^+_{acq2} - CS2^-_{acq2}$).

These manipulation checks were tested with planned comparisons (one-tailed for prediction a and b; two-tailed for prediction c).

2.4.5.1.1 US-expectancy

a. US expectancy ratings in the last acquisition block increased more during reinforced stimuli (CS^+, $CS1^+$, $CS2^+$) compared the unreinforced stimuli (CS^-, $CS1^-$, $CS2^-$), $F(1, 38) = 290$, $p < .001$.

b. Participants increased their US expectancy for each of the reinforced stimuli compared to their respective control stimuli in the last acquisition block: CS^+ compared to CS^-: $F(1, 38) = 349.88$, $p < .001$; $CS1^+$ versus $CS1^-$: $F(1, 38) = 104.11$, $p < .001$; $CS2^+$ versus $CS2^-$: $F(1, 38) = 171.91$, $p < .001$.

c. We observed no significant differences in strength of acquisition between the three pairs of CSs: (CS^+ - CS^-) versus ($CS1^+$ - $CS1^-$), $F(1, 38) = .01$, $p = .92$; (CS^+ - CS^-) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = .54$, $p = .467$; ($CS1^+$ - $CS1^-$) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = .18$, $p = .678$.

See table 1.

2.4.5.1.2 Startle eyeblink

a. CS-ITI difference scores indicated that the startle eye blink was potentiated more to reinforced compared to unreinforced stimuli during acquisition, $F(1, 38) = 23.89$, $p = .001$.

b. All reinforced stimuli tended to evoke a higher startle response compared to their respective control stimuli: CS^+ versus CS^-: $F(1, 38) = 6.42$, $p = .016$; $CS1^+$ versus $CS1^-$: $F(1, 38) = 3.33$, $p = .076$; $CS2^+$ versus $CS2^-$: $F(1, 38) = 15.45$, $p < .001$.

c. The three CS pairs did not differ significantly in their strength of differential learning: (CS^+ - CS^-) versus ($CS1^+$ - $CS1^-$), $F(1, 38) = .04$, $p = .85$; (CS^+ - CS^-) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = 2.44$, $p = .13$; ($CS1^+$ - $CS1^-$) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = 2.46$, $p = .12$.

See figure 2.

2.4.5.1.3 Skin conductance

a. As expected, SCRs to the reinforced stimuli were significantly higher than those to the unreinforced stimuli during the second acquisition block, $F(1, 38) = 14.48$, $p < .001$.

b. Each of the reinforced stimuli evoked higher skin responses in the second acquisition block than their control stimuli: CS^+ compared to CS^-: $F(1, 38) = 10.87$, $p = .001$; $CS1^+$ versus $CS1^-$: $F(1, 38) = 3.66$, $p = .031$; $CS2^+$ versus $CS2^-$: $F(1, 38) = 5.67$, $p = .011$.

c. The three CS pairs did not differ significantly in their strength of differential learning:: (CS^+ - CS^-) versus ($CS1^+$ - $CS1^-$), $F(1, 38) = 2.35$, $p = .134$; (CS^+ - CS^-) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = .99$, $p = .327$; ($CS1^+$– $CS1^-$) versus ($CS2^+$ - $CS2^-$), $F(1, 38) = .55$, $p = .464$.

See table 1.

2.4.5.1.4 Respiratory rate

a. In the last acquisition block, participants tended to reduce their respiratory rate less in response to the CS^+s compared to the CS^-s, $F(1, 35) = 2.804$, $p = .051$.

b. However, this differential effect did not reach significance for each CS pair separately: CS^+ versus CS^-: $F(1, 35) = 2.372$, $p = .065$; $CS1^+$ versus $CS1^-$: $F(1, 35) = 4.02$, $p = .026$; $CS2^+$ versus $CS2^-$: $F(1, 35) = .083$, $p = .385$.

c. The + versus - contrasts for the three CS pairs did not differ significantly from each other: (CS^+ - CS^-) versus ($CS1^+$ - $CS1^-$), $F(1, 35) = .04$, $p = .835$; (CS^+ - CS^-) versus ($CS2^+$ - $CS2^-$), $F(1, 35) = 1.98$, $p = .168$; ($CS1^+$ - $CS1^-$) versus ($CS2^+$ - $CS2^-$), $F(1, 35) = 2.83$, $p = .102$.

See table 1.

2.4.5.1.5 Tidal Volume

a. During the second acquisition block, tidal volume increased less in response to the CS^+s compared to the CS^-s,, $F(1, 35) = 4.53$, $p = .02$.

b. However, the differential effect was significant for the CS and the CS1 pairs, whereas not for the CS2 pair: CS^+ versus CS^-: $F(1, 35) = 3.72$, $p = .03$; $CS1^+$ versus $CS1^-$: $F(1, 35) = 5.08$, $p = .015$; $CS2^+$ versus $CS2^-$: $F(1, 35) = .057$, $p = .406$.

c. The + versus - contrasts for the three CS pairs did not differ significantly from each other: $(CS^+ - CS^-)$ versus $(CS1^+ - CS1^-)$, $F(1, 35) = .089$, $p = .768$; $(CS^+ - CS^-)$ versus $(CS2^+ - CS2^-)$, $F(1, 35) = 1.15$, $p = .291$; $(CS1^+ - CS1^-)$ versus $(CS2^+ - CS2^-)$, $F(1, 35) = .744$, $p = .394$. See table 1.

2.4.5.2 Fear Extinction.

We expected that at the end of extinction, differences between CS^+ and CS^- would be no longer significant: $CS^+_{ext2} = CS^-_{ext2}$

2.4.5.2.1 US-expectancy

At the end of extinction, US-expectancy ratings changed similarly during the CS+ compared to the CS-, or, the CS^+/CS^- difference was no longer significant in the last block of extinction: $F(1, 38) = 0.55$, $p = .23$. See table 1.

2.4.5.2.2 Startle eyeblink

As predicted, no significant differences were observed between CS^+ and CS^- (CS-ITI difference scores) in the second extinction block, $F(1, 38) = .15$, $p = .349$, while in the

first extinction block, a fear potentiated startle to CS^+ relative to CS^- was still present: $F(1, 38) = 4.13$, $p = .024$. See figure 2.

2.4.5.2.3 Skin conductance

In line with our prediction, the CS^+/CS^- difference was not significant in the second block of extinction: $F(1, 38) = 0.02$, $p = .42$. See table 1.

2.4.5.2.4 Respiratory rate

In the second block of extinction the pattern installed during acquisition was still present: participants reduced their respiratory rate more to the CS^- compared to the CS^+ , $F(1, 35) = 4.87$, $p = .016$. See table 1.

2.4.5.2.5 Tidal volume

During the second block of extinction, no significant differences in the tidal volume response were observed for CS^+ compared to CS^-, $F(1, 35) = .04$, $p = .424$. See table 1.

3. Results

3.1 Spontaneous recovery

3.1.1 US-expectancy

a. Participants increased their US-expectancy ratings significantly more to the CS^+ relative to the CS^- in the first test block compared to the last extinction block: $(CS^+_{ext2} - CS^-_{ext2}) < (CS^+_{test1} - CS^-_{test1})$: $F(1, 38) = 4.06$, $p = .025$.

b. In addition, a differential effect was reinstalled on day 2, as the CS^+ evoked a stronger increase of US expectancy than the CS^- in the first test block, $F(1, 38) = 5.46$, $p = .012$.

Both these results demonstrate spontaneous recovery of US expectancy.

See figure 1.

3.1.2 Startle eyeblink

a. In contrast to what figure 2 seems to suggest, startle potentiation to the CS+ relative to the CS- was not greater during the test phase compared to the last extinction block: ($CS^{+}_{ext2} - CS^{-}_{ext2}$) versus ($CS^{+}_{test} - CS^{-}_{test}$), $F(1, 38) = 1.50$, $p = .114$.

b. The (CS-ITI)-values of CS^{+} and CS^{-} were not significantly different from each other during test, $F(1, 38) = 1.14$, $p = .146$.

See figure 2.

3.1.3 Skin conductance

a. In contrast to what figure 2 seems to suggest, the CS did not evoke a significantly greater differential skin response during the first block of the test phase compared to the last block of extinction, $F(1, 38) = 1.59$, $p = .10$.

b. A near significant higher SCR was visible during the first test block in response to the CS^{+} compared to the CS^{-}, $F(1, 38) = 2.33$, $p = .067$.

See figure 3.

3.1.4 Respiratory rate

a. Participants did not reduce their respiratory rate more to the CS^{-} relative to the CS^{+} in the first test block compared to the last extinction block: ($CS^{+}_{ext2} - CS^{-}_{ext2}$) versus ($CS^{+}_{test1} - CS^{-}_{test1}$), $F(1, 35) = .001$, $p = .47$.

b. Also in the first test phase no significant differences were observed in respiratory rate between CS^{+}_{test1} and CS^{-}_{test1}, $F(1, 35) = 1.85$, $p = .09$.

See table 1.

3.1.5 Tidal volume

a. The differential effect for the CS pair tended to be smaller during the last extinction block than during the first test block: $(CS^{+}_{ext2} - CS^{-}_{ext2}) < (CS^{+}_{test1} - CS^{-}_{test1})$, $F(1, 35) = 2.59$, $p = .055$.

b. Data of the first test block show a higher increase in Vt in response to the CS^{-} than to the CS^{+}, $F(1, 35) = 5.23$, $p = .014$.

See table 1.

3.2 Retention of generalization of fear extinction.

3.2.1 US-expectancy

a. A greater differential effect was observed for the to-be-extinguished CS-pair during the first extinction block compared to the unextinguished CS-pairs during the first block of test: $(CS^{+}_{ext1} - CS^{-}_{ext1}) > (CS1^{+}_{test1} - CS1^{-}_{test1}; CS2^{+}_{test1} - CS2^{-}_{test1})$, $F(1, 38) = 28.43$, $p < .001$.

b. In the first block of the test phase, a differential effect was still present for the unextinguished CS-pairs: participants increased their US-expectancy more to the $CS1^{+}$ and $CS2^{+}$ compared to the $CS1^{-}$ and $CS2^{-}$: $F(1, 38) = 19.51$, $p < .001$.

See figure 1.

3.2.2 Startle eyeblink

a. The differential effect in startle potentiation for the to-be-extinghuished CS-pair in the first extinction block was not significantly larger than for the unextinguished CS-pairs in

the first test block: (CS^{+}_{ext1} – CS^{-}_{ext1}) > ($CS1^{+}_{test}$ – $CS1^{-}_{test}$; $CS2^{+}_{test}$ – $CS2^{-}_{test}$), $F(1, 38) = .20$, $p = .328$,

b. During the test phase, startle blinks were potentiated during $CS1^{+}_{test}$ and $CS2^{+}_{test}$ compared to $CS1^{-}_{test}$ and $CS2^{-}_{test}$, $F(1, 38) = 7.82$, $p = .008$.

See figure 2.

3.2.3 Skin conductance

a. Differential SCRs for CS1 and CS2 during the first test block were not significantly smaller than the CS+/CS- difference in the first extinction block, $F(1, 38) = 1.18$, $p = .145$.

b. SCRs to $CS1^{+}$ and $CS2^{+}$ were not higher compared to SCRs to $CS1^{-}$ and $CS2^{-}$ in the first test block: $F(1, 38) = .64$, $p = .215$.

See figure 3.

3.2.4 Respiratory rate

Since no equal levels of fear acquisition to the reinforced stimuli were obtained in respiratory rate, this hypothesis cannot be tested.

3.2.5 Tidal volume

Since no equal levels of fear acquisition to the reinforced stimuli were obtained in tidal volume, this hypothesis cannot be tested.

3.3 Perceptual gradient of generalization of fear extinction.

3.3.1 US-expectancy

During the first block of the test phase, the differential effect for the CS1 pair was not significantly smaller than for the CS2 pair: $F(1, 38) = .69$, $p = .255$. Thus, in contrast to

what Figure 1 suggests, the perceptual gradient of generalization of fear extinction was not significantly present in the US expectancy ratings.

3.3.2 Startle eyeblink

During test, startle potentiation to CS2+ relative to CS2- was not greater than startle potentiation to CS1+ relative to CS1-: $F(1, 38) = .226$, $p = .32$. See figure 2.

3.3.3 Skin conductance

In contrast to what Figure 2 seems to suggest, +/- differences for CS1 were not significantly smaller than for CS2 during the first test block, $F(1, 38) = .72$, $p = .20$. See figure 3.

3.3.4 Respiratory rate

Since no equal levels of fear acquisition to the reinforced stimuli were obtained in respiratory rate, this hypothesis cannot be tested.

3.3.5 Tidal volume

Since no equal levels of fear acquisition to the reinforced stimuli were obtained in tidal volume, this hypothesis cannot be tested.

Insert Figure 1, 2 and 3 here

4. Discussion

With the current study we aimed to examine the generalization of fear extinction after a retention interval of one day. To this end, three geometrical figures of different sizes (CS^+, $CS1^+$ and $CS2^+$) were reinforced by a short-lasting suffocation experience (US), and three other geometrical figures (CS^-, $CS1^-$, $CS2^-$) remained unreinforced during a fear acquisition phase on day one. Next, only the CS^+ was extinguished by presenting it in the absence of the US. The following day, fear responses to all stimuli were assessed without any US-presentation (test phase).

Our findings confirm that, except for CS2 in the respiratory measures, equal levels of fear were installed and subsequently extinguished to the reinforced relative to the unreinforced stimuli on day 1. As such, the present findings convincingly show that the present paradigm with a panic-relevant, respiratory US is apt to study fear conditioning phenomena in a highly-controlled manner. Fear conditioning was established and subsequently extinguished in all traditional outcome measures including US expectancy, startle potentiation and skin conductance. In addition, participants also learned to breathe faster and more shallow in anticipation of the suffocation experience (US). The nature of the conditioned respiratory response in the present study is in line with findings from Van Diest et al. (2009) who observed a conditioned increase in respiratory rate when using an electrical shock as US. As such, these findings confirm again that breathing behavior is susceptible to fear conditioning processes. This calls for the inclusion of respiratory parameters in the experimental study of fear learning since fear conditioning of respiratory behavior could be an important element in the progression and maintenance of clinical anxiety.

On day 2, we expected to observe spontaneous recovery of the CR to the CS^+ (a priori hypothesis 1), as numerous studies in the fear conditioning literature have reported on return of fear with the mere passage of time (Bouton, 2002; Vervliet et al., 2013; McConnell, 2014). In addition, temporal closeness of acquisition and extinction, as present in this study, is known to promote spontaneous recovery (Maren, 2014). Consistent with our hypotheses, we observed spontaneous recovery for the US-expectancy ratings (see Fig 1) and for tidal volume (see table 1). The differential effect that was extinguished on day one in both these measures was reinstalled during the test at day two (hypothesis1b), and it was bigger during test compared to the end of extinction (hypothesis 1a). Skin conductance tended to follow this pattern, as both contrasts were marginally significant (see Fig 2). Although figure 3 suggests that also a differential return of startle potentiation was present for the CSs during test, these contrasts were not significant. Compared to US-expectancy and SCR, startle response data are more variable and it is recommended to average across 4-5 startle responses to obtain a reliable estimation of startle potentiation (Blumenthal et al., 2005). However, as return of fear in the laboratory is typically a short-lived phenomenon only showing up in the first trial(s)it is possible that startle EMG is not sensitive enough to pick up on short-lived phenomena such as return of fear.

Hypothesis 2a and 2b aimed to test the retention of generalization of fear extinction. Retention of generalization of fear extinction was most clearly present in the US-expectancy ratings. Although a differential effect was still present for CS1 and CS2 during the first test block (hypothesis 2b), this differential effect was reduced compared to the one observed for the CSs at the start of the extinction phase (hypothesis 2a). Thus, despite the fact that $CS1^+$ and $CS2^+$ had never been presented unreinforced (without the US), they evoked significantly lower levels of fear the day after an extinction procedure

with a perceptually similar stimulus (see Fig 1). This finding corroborates findings of some older studies that acquired generalization of fear extinction, also after a retention interval of 24 hours (Bass and Hull, 1934; Hovland, 1937). They seem however at odds with data of Vervliet and Geens (2014) who obtained only limited fear reduction to a CS^+ after an extinction procedure with a stimulus that differed in only one perceptual feature with the CS^+ (e.g. the colour). Vervliet and Geens (2014) noted however that their extinction data have to be interpreted with caution since only limited reduction of the CR was obtained during fear extinction. However, also in an older study, Vervliet et al. (2004) found no evidence for solid fear extinction generalization and concluded that fear extinction is highly restricted to the specific features of the extinction stimulus. A critical difference between the studies of Vervliet et al. (2004, 2014) and our study is that the former authors assessed fear responses to the non-extinguished stimulus immediately after extinction, that is, during the fear acquisition and fear extinction memory consolidation window. In contrast, participants in our study had to retrieve the extinction memory from long-term memory at the moment of testing. It has been demonstrated that the presence of stress has a differential influence on memory encoding versus memory retrieval (Schwabe et al., 2011; Qin et al., 2012). While stress enhances encoding, it impairs retrieval. Stress during acquisition may have promoted the encoding of detailed stimulus features and contingencies in both experiments. Such detailed encoding may have limited generalization of extinction in the study of Vervliet et al. (2014), where generalization of extinction was tested during the consolidation window. In contrast, stress may have hindered the retrieval of detailed stimulus features, leading to more liberal generalization of extinction.

Retention of generalization of fear extinction was less convincing for the psychophysiological outcomes. Whereas it was clearly absent for startle EMG, our

findings more or less support retention of generalization of fear extinction for SCR. Although a differentially conditioned response was established for SCR in late acquisition, this differential response had disappeared for CS1 and CS2 one day later during test (hypothesis 1b), despite the fact that *not* the $CS1^+$ and $CS2^+$ but only the perceptually similar CS^+ had been extinguished prior to the test. This pattern may reflect a very strong (retention of) generalization of fear extinction, to the extent of complete extinction of the differential response to CS1 and CS2. Alternatively, such pattern could also arise from a failure to retrieve the fear memory for $CS1^+$ and $CS2^+$ at day two. Hypothesis 2a was not confirmed for SCR, but this is likely due to the fact that complete extinction already occurred in the first extinction block (see Figures 2), rendering contrast 2a invalid as a test for the retention of generalization of fear extinction.

Finally, in contrast with findings from Hovland (1937), we found no evidence for the retention of a perceptual fear extinction gradient (hypothesis 3). Our study may have been underpowered to detect such effect, as the visual pattern of the US-expectancy, startle and SCR (see Fig 1, 2 and 3) do suggest such gradient.

Overall, the hypothesized patterns showed up much clearer for the US-expectancy ratings than for the psychophysiological outcomes. This may be related to habituation in physiological responding, which may easily lead to floor effects at the moment the critical tests of retention of generalization of fear extinction were tested.

Apart from this potential limitation, our study is characterized by some other limitations. First, we only assessed generalization of fear extinction at the retention test. As such, we cannot sort out whether the lack of a perceptual gradient in the retention of generalization of fear extinction (hypothesis 3) is due to the retention interval or to the absence of such gradient at day one. Future research may want to assess generalization of fear extinction also immediately after extinction, although this may lengthen the

procedure a lot and may cause unintended, direct (instead of generalized) extinction to the generalization stimuli.

Second, it not unconceivable that also spontaneous recovery of fear to the CS+ may have generalized to the generalization stimuli in the current study. Thus, we cannot exclude that $CS1^+$ responses were differently affected by spontaneous recovery than $CS2^+$ responses. The net differences of CRs to these stimuli might be due both to the generalization gradient of extinction along the perceptual continuum and to a generalization gradient of spontaneous recovery. Further research could address this issue, for example by using an ABB-design, introducing a context change between acquisition and extinction, instead of the AAA-design we applied. Research has shown that testing in the same context as extinction, but different from acquisition, reduces spontaneous recovery (Johnson, Escobar and Timble, 2010). Also, increasing the time between acquisition and extinction should reduce spontaneous recovery (Maren, 2014).

Third, we did not use a standardized clinical interview to detect and exclude participants with substance use or anxiety disorders from the study.

Fourth, future research may want to include a control group that did not receive any extinction. When fear responses to the CS^+s during test of such control group exceed those of the other group who received extinction to one of the CS^+s, one could conclude with greater confidence that generalization of fear extinction took place in the latter group.

A last limitation of this study is the lack of self-reported measures of anxiety. Future research should not only comprise the registration of baseline anxiety to control for its influence on fear conditioning processes, but also self-reported valence and arousal of the CSs and the US could be very informative.‘

In summary, our results demonstrate that generalization of fear extinction can be obtained and retained over time. This was shown convincingly for self-reported US-expectancy in particular. Although visual inspection of the data also suggested the presence of a perceptual gradient in the generalization of fear extinction, the present data failed to provide statistical evidence for the existence of such gradient.

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